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# Northeastern University

360 Huntington Avenue, Boston, Massachusetts 02115

Pharmacy and Allied Health Professions  
Section of Medicinal Chemistry



June 14, 1984

RECEIVED

JUN 25 1984

SECTION 626

Jet Propulsion Laboratory  
California Institute of Technology  
4800 Oak Grove Drive  
Pasadena, CA 91103

ATTN: Dr. Dorothy Quinlan  
Sr. Contract Negotiator

SUBJECT: Final Report on Subcontract 956837, "Development of a New Class of Chemical and Biological Ultrasensors: Ribonuclease Contamination and Control"

## A. Ribonuclease Contamination

In order to define ribonuclease contamination, an assay for ribonuclease having picogram level sensitivity was established as defined by the scheme shown in Figure 1. In this assay, polycytidylic acid is digested by ribonuclease leading to smaller fragments of poly C that remain soluble after treatment of the sample with perchloric acid and lanthanum acetate. An absorbance measurement at 260 nm of the supernatant from the centrifuged sample measures the ribonuclease. A standard curve is shown in Figure 2.

Using this assay procedure, ribonuclease contamination was found to be significant in routine laboratory proteins, in particular, bovine serum albumin, lysozyme, catalase, and cytochrome C. This was confirmed by demonstrating a considerable reduction in this activity in the presence of phosphate buffer since phosphate inhibits ribonuclease. Ribonuclease contamination was not significantly encountered in routine laboratory glassware, plasticware, column surfaces, chromatographic particles, and buffer reagents, including airborne contamination. Some contamination could be introduced by fingerprints, however.

## B. Chemical and Physical Techniques for Overcoming Ribonuclease Contamination

Ribonuclease contamination in routine laboratory proteins was overcome, at least below the sensitivity level of our assay, by treating the sample with iodoacetate or iodoacetamide, especially at an elevated temperature of 60-80 °C, either in a routine buffer or in the presence of 6 M urea or 6 M guanidine hydrochloride. This technique was quite powerful, eliminating ribonuclease activity even

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(NASA-CR-175466) DEVELOPMENT OF A NEW CLASS  
OF CHEMICAL AND BIOLOGICAL ULTRASENSORS:  
RIBONUCLEASE CONTAMINATION AND CONTROL  
Final Report (Northeastern Univ.) 4 P  
HC A02/MF A01

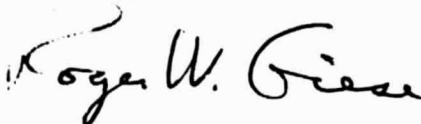
Dr. Quinlan

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starting with a sample of ribonuclease itself. Ribonuclease contamination in fingerprints was overcome either by good laboratory technique, avoiding fingerprint contamination of equipment surfaces, or by heating the contaminated surfaces to 200 °C, or by washing the contaminated surface with 0.2% diethylpyrocarbonate in water.

Yours truly,

A handwritten signature in dark ink, appearing to read "Roger W. Giese". The signature is fluid and cursive, with the first name "Roger" being the most prominent.

Roger W. Giese, Ph.D.  
Professor  
Clinical Chemistry

RWG:cma

cc: R. McNeil, Jr.  
I. Skurnick

# RIBONUCLEASE ASSAY PROCEDURE

RNASE + POLY C

pH 7.5 ↓ 37°C

NUCLEOTIDES + POLY C +  
RNASE

HClO<sub>4</sub> ↓ LaOAc

ACID SOLUBLE NUCLEOTIDES

1:10 ↓ H<sub>2</sub>O

ABSORBANCE 260 NM

FIGURE 1

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